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09/520,538	03/08/2000	Arlene A. Wise	S-91,714	2050

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EXAMINER	
STEADMAN, DAVID J	
ART UNIT	**PAPER NUMBER**
1652	7

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Please find below and/or attached an Office communication concerning this application or proceeding.

PTO-90C (Rev. 07-01)

Office Action Summary	Application No. 09/520,538	Applicant(s) WISE ET AL.	
	Examiner David J. Steadman	Art Unit 1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____ .

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1 and 8* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1 and 8* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☒ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

Status of the Application

Claims 1 and 8 are pending in the application.

Applicants' amendment to claim 1, cancellation of claims 2-7, and addition of claim 8 in Paper No. 6, filed 10/09/01 is acknowledged.

Applicants' arguments in Paper No. 6 have been fully considered and are deemed to be persuasive to overcome some of the rejections previously applied. Rejections and/or objections not reiterated from previous office actions are hereby withdrawn.

The text of those sections of Title 35 U.S. Code not included in the instant action can be found in a prior Office action.

Oath/Declaration

1. The oath or declaration remains defective. It is noted that applicants agree to submit a corrected declaration upon allowance of claimed subject matter.

Sequence Compliance

2. This application is not sequence compliant as stated in Paper No. 4. It is noted that applicants agree to submit a computer-readable form and paper copy of the sequence listing and a statement of identical content upon allowance of claimed subject matter. **Applicants should note that compliance with the sequence rules is not a deferable requirement and a Notice of Allowance cannot be issued until the requirement is satisfied.**

Specification/Informalities

3. The drawings remain objected to by the Examiner because of the following informalities: the Y-axes of Figures 2-7 are not labeled. It is noted that applicants agree to submit corrected drawings upon allowance of claimed subject matter.

Claim Rejections - 35 USC § 103

4. Claims 1 and 8 are rejected under 35 U.S.C. 103(a) as being unpatentable over Shingler et al. (J Bacteriol 176:7550-7557) in view of any of Willardson et al. (Appl Environ Microbiol 64:1006-1012), Schirmer et al. (J Bacteriol 179:1329-1336), Ng et al. (J Bacteriol 177:1485-1490), Burchhardt et al. (Mol Gen Genet 254:539-547), or Byrne et al. (J Bacteriol 178:6327-6337) and either of Cadwell et al. ("Mutagenic PCR" pp 583-589 in "PCR Primer, A Laboratory Manual", Cold Spring Harbor Laboratory Press, 1995) or Stemmer (Nature 370:389-391). Claims 1 and 8 are drawn to a method of enhancing a response of *Pseudomonas putida, Acinetobacter,* and *Escherichia coli* bacteria to phenols and substituted phenols said bacteria having a regulatory protein selected from DmpR, MopR, PhhR, PhlR, XylR, and TbuT with a sensor domain for detecting phenols, a DNA binding region, and a transcriptional activation region, the method comprising the steps of removing the DNA encoding the sensor domain, subjecting the sensor domain DNA to mutagenic PCR or gene shuffling, ligating the mutant sensor domain into the DNA encoding the regulatory protein, and testing the bacteria for enhanced response to said phenols.

Shingler et al. teach that the A domain, i.e., sensor domain, of DmpR binds (methyl)phenols resulting in transcriptional activation (page 7550, abstract and page 7556, paragraph 2) and that DmpR responds to (methyl)phenols with the magnitude of transcriptional response differing depending on the position of the methyl substituent (page 7550, abstract) and further teach that the response to *para*-substituted phenolic compounds (i.e., 4-methylphenol and 3,4-methylphenol) is relatively poor (page 7550, Introduction). Shingler et al. further teach a method of mutating DmpR by chemical mutagenesis (pages 7551-7552 under *Construction of P_o Km selection strain and isolation of DmpR specificity mutant*) to generate a mutant DmpR that, when expressed in *P. putida* with a chromosomally inserted reporter gene (page 7552, under *Construction of P_o luxAB reporter strain and luciferase assays*), exhibits increased luciferase expression relative to wild-type DmpR in response to 4-methylphenol, 3,4-dimethylphenol, and 4- ethylphenol (page 7554, Fig 3) and that sequencing the gene encoding the DmpR mutant revealed a mutation at codon 135 (page 7554, under *Genetic selection of an effector specificity*

mutant, DmpR-E135K) of the A domain of DmpR (amino acids 1-211; page 7556, paragraph 2). Shingler et al. also teach that a comparison of the responses of the wild-type and mutant DmpR to various phenolic derivatives suggests that, in addition to the increased responses of the mutant to 4-methylphenol, 3,4-dimethylphenol, and 4-ethylphenol, the mutant DmpR mediates responses to phenol, 2-methylphenol, and 3-methylphenol to similar extents as wild-type DmpR (page 7554, under *Effector profile comparison of DmpR$^+$ and DmpR-E135K*), suggesting that the mutant DmpR response to 4-methylphenol, 3,4-dimethylphenol, and 4-ethylphenol is enhanced relative to wild-type DmpR without altering the function of the other domains (DNA binding domain and transactivation domain). Shingler et al. further teach that DmpR shares significant sequence similarity with XylR, a *Pseudomonas* regulator of toluene and xylene catabolism (page 7550, Introduction, paragraph 1) and that mutations at residues 135 and 172 of DmpR and XylR, respectively, result in the ability of the proteins to recognize a novel effector compound (page 7556, left column, bottom).

Shingler et al. *do not teach* a method for enhancing the response of *Acinetobacter* or *Escherichia coli* to phenols and substituted phenols by removing the DNA encoding the sensor domain of DmpR, MopR, PhhR, PhlR, XylR, or TbuT, mutating the sensor domain by mutagenic PCR or gene shuffling, ligating the mutant sensor domain into the DNA encoding the regulatory protein, and testing the bacteria for enhanced response to said phenols.

Willardson et al. teach a biosensor using *Escherichia coli* expressing XylR that responds to toluene and derivatives thereof by luminescence proportional to the concentration of toluene or derivatives thereof present in a medium. Willardson et al. further teach "the development of this biosensor for toluene and its derivative compounds demonstrates the feasibility of constructing similar biosensors with specificity for other organic contaminants by using their corresponding transcriptional activators" (page 1011, bottom – 1012, top). Willardson et al. also suggest using other bacterial strains as biosensors (page 1012, top).

Schirmer et al. teach MopR is regulator protein of phenol metabolism in *Acetobacter calcoaceticus* (page 1329, Introduction) and teach characterization of MopR response to various effector compounds

(page 1332, Fig 3), the results of which suggest that MopR exhibits a response to 3,4-dichlorophenol, while DmpR does not respond to the same compound (page 1334, right column). Schirmer et al further teach the A domain of MopR consists of residues 1-150 (page 1333, left column).

Ng et al. teach PhhR is regulator protein of phenol and monomethylated phenol metabolism in *Pseudomonas putida* (page 1485, Introduction) and teach characterization of PhhR response to various effector compounds (page 1489, Fig 4). Ng et al further teach the A domain of PhhR consists of residues 1-210 (page 1487, right column).

Burchhardt et al. teach PhlR is a regulator protein of phenol metabolism in *P. putida* (page 539, Abstract) and disclose the nucleotide sequence and a method of expressing PhlR in bacteria (page 540-541 under Materials and Methods). Burchhardt et al. also teach that PhlR shares relatively high homology with XylR, PhhR, and DmpR (page 542, left column).

Byrne et al. teach TbuT is a regulator protein of toluene metabolism in *Burkholderia pickettii* (page 6327, Introduction) and teach characterization of TbuT response to various effector compounds (page 6333, Fig 6), the results of which suggest that TbuT exhibits a response to trichloroethylene (page 1334, right column), and disclose that this is the first regulator protein that responds to trichloroethylene (page 6336, right column). Byrne et al further teach the A domain of TbuT consists of residues 1-238 (page 6333, Fig 5).

Cadwell et al. teach a mutagenic PCR method of randomly mutating a nucleic acid in order to generate a library of mutant nucleic acids (page 584 under *Protocol*). Cadwell et al. further teach that using these mutants, one can apply a screening method to isolate individual clones that exhibit a particular property. (page 583, Introduction, paragraph 2).

Stemmer et al. teach a method of *in vitro* homologous recombination of pools of selected mutant genes by random fragmentation and PCR reassembly, i.e., gene shuffling (page 390, Fig 1) and teach that one would use gene shuffling over mutagenic PCR because mutagenic PCR is not combinatorial and thus, is more limited in the number of possible mutations (page 389, abstract and page 390, right column).

Also, at the time of the invention, it would have been obvious to one of ordinary skill in the art to mutate *only* the sensor domain, i.e., the A domain as described by Shingler et al., because one of ordinary skill would have recognized that, in order to broaden the binding specificity of the sensor domain, one need mutate *only* the domain responsible for binding the effector compound, i.e., the sensor domain, and not the DNA binding or transactivation domains.

Therefore, it would have been obvious to one of ordinary skill in the art to combine the teachings of Shingler et al. with any of Willardson et al., Schirmer et al., Ng et al., Burchhardt et al., and Byrne et al. and Cadwell et al. or Stemmer et al. for a method of enhancing the response of *P. putida, Acinetobacter, and Escherichia coli* to phenols and substituted phenols by removing the DNA encoding the sensor domain of DmpR, MopR, PhhR, PhlR, XylR, or TbuT, mutating the sensor domain by mutagenic PCR or gene shuffling, ligating the mutant sensor domain into the DNA encoding the regulatory protein, and testing the bacteria for enhanced response to said phenols. One would have been motivated to mutate *only* the sensor domain of the regulator proteins of claim 1 because of the teachings of Shingler et al. who taught that the binding specificity of regulatory proteins can be broadened by mutating only the sensor domain and not the DNA binding and transcriptional activation domains. One would have been motivated to use other bacteria for regulator protein expression because of the teachings of Willardson et al. who taught other biosensors can be generated with specificity for other organic contaminants under varying conditions. One would have been motivated to use mutagenic PCR or gene shuffling to mutate the sensor domain because of the teachings of Cadwell et al. who taught that by using mutagenic PCR, one can create a library of mutants to isolate those with desirable properties or because of the teachings of Stemmer et al. who taught that by using gene shuffling, one can create a combinatorial library of mutants. One would have a reasonable expectation of success for a method of enhancing the response of *P. putida, Acinetobacter, and Escherichia coli* to phenols and substituted phenols by removing the DNA encoding the sensor domain of DmpR, MopR, PhhR, PhlR, XylR, or TbuT, mutating the sensor domain by mutagenic PCR or gene shuffling, ligating the mutant sensor domain into the DNA encoding the regulatory protein, and testing the bacteria for enhanced response to said phenols

because of the results of Shingler et al., Willardson et al., Schirmer et al., Ng et al., Burchhardt et al., Byrne et al., and Cadwell et al. or Stemmer et al. Therefore, claims 1 and 8, drawn to a method of enhancing the response of *P. putida, Acinetobacter, and Escherichia coli* to phenols and substituted phenols by removing the DNA encoding the sensor domain of DmpR, MopR, PhhR, PhlR, XylR, or TbuT, mutating the sensor domain by mutagenic PCR or gene shuffling, ligating the mutant sensor domain into the DNA encoding the regulatory protein, and testing the bacteria for enhanced response to said phenols would have been obvious to one of ordinary skill in the art.

Applicants argue that Shingler et al. teach a method of increasing growth of *E. coli* using substituted phenols as a carbon source by increasing *dmp* copy number and a method of mutating DmpR using chemical mutagenesis that resulted only in a single point mutation in the sensor domain. Applicants argue that the method of mutation as taught by Shingler et al. could have produced mutants with a decreased ability to utilize (methyl)phenols. Applicants' argument is not found persuasive. While Shingler et al. suggest increasing *dmp* copy number as one mechanism of increasing utilization of (methyl)phenols by *P. putida*, Shingler et al. suggest that this method is not optimal and suggest instead using a mutant DmpR in order to increase the response to (methyl)phenols while maintaining the wild-type response to phenol and 2-mp (page 7554, under *Genetic selection of an effector specificity mutant, DmpR-E135K*). The method of mutating the sensor domain of DmpR of Shingler et al. positively demonstrated that by using chemical mutagenesis, one can isolate a mutant sensor domain with increased response to (methyl)phenol effector compounds with an unaltered response to other phenolic compounds (see Fig 3, page 7554 of Shingler et al.). Furthermore, while applicants' method of generating such mutants may generate a greater number of amino acid mutations within the sensor domain compared with the methods of the prior art, the instant claims are drawn to methods that are suggested by the combined references of Shingler et al., Willardson et al., Schirmer et al., Ng et al., Burchhardt et al., Byrne et al., and Cadwell et al. or Stemmer et al. and the number of point mutations generated by applicants' claimed methods is not commensurate in scope with the instant claims.

Applicants further argue that, based on the examiner's comments regarding the unpredictability of the functional effect(s) of mutations within a protein's amino acid sequence in the enablement rejection made under 35 USC 112, 1st paragraph, one would not be motivated to combine the cited references in order to practice the claimed invention and therefore, the examiner has failed to meet the burden of obviousness under 35 USC 103(a). Applicants' argument is not found persuasive. It is noted that the statement regarding the unpredictability of the functional effect(s) of mutations within a protein's amino acid sequence included in the enablement rejection of Paper No. 4 was directed to applicants' broadest embodiments of original claims 1 and 4-7 that were not enabled by the instant specification, i.e., a method of enhancing *any* response of *any* bacteria to *any* organic molecules, said bacteria having *any* regulatory protein with *any* sensor domain that binds to *any* cognate promoter sequence and activates expression of *any* genes encoding metabolic enzymes, said method comprising performing *any* modification to *any* sensor domain of *any* regulatory protein. The rejection of original claims 1-7 under 35 USC 103(a) was directed to applicants *specific* embodiments that were recited in claims 2 and 3, i.e., a method of enhancing the response of *Pseudomonas* and *Acinetobacter* bacteria to phenols or substituted phenols, said bacteria expressing a regulatory protein from the group consisting of DmpR, MopR, PhhR, PhlR, XylR, and TbuT by mutagenic PCR or gene shuffling. Therefore, based on the teachings of Shingler et al., Willardson et al., Schirmer et al., Ng et al., Burchhardt et al., Byrne et al., and Cadwell et al. or Stemmer et al., one would have been motivated to mutate *specific* sensor domains of DmpR, MopR, PhhR, PhlR, XylR, and TbuT by mutagenic PCR or gene shuffling in order to improve the response of bacteria to phenols as encompassed by claims 1 and 8 as described above. Therefore, the cited references combine to make obvious applicants' methods of claims 1 and 8.

Conclusion

5. No claim is in condition for allowance. All claims are rejected.

Applicants' amendment to claim 1 and addition of claim 8 necessitated the new grounds of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to David Steadman, whose telephone number is (703) 308-3934. The examiner can normally be reached Monday-Friday from 8:00 am to 4:30 pm. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathapura Achutamurthy, can be reached at (703) 308-3804. The FAX number for this Art Unit is (703) 308-4242. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Art Unit receptionist whose telephone number is (703) 308-0196.

David J. Steadman, Ph.D.

Rebecca Prouty

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